Nutritional Evaluation of Milk Processed for Removal of Cationic Radionuclides. Chemical Analyses

Chemical constituents of milk, which had been treated with Amberlite IR-120 resin, in the CaMg-KNa cycle have been studied. Comparisons of results obtained on control and resin-treated milk samples showed no significant change in total solids, butterfat, protein, flavor quality, carotene, vitamin A, riboflavin, pantothenic acid, folic acid, or vitamin B_{12}. The ash, potassium, and citric acid contents of the resin-treated milk increased approximately 14, 80, and 100%, respectively, compared with control milk. The thiamine, niacin, and vitamin B_6 contents of the processed milk decreased 50, 27, and 15%, respectively. The data indicate that 86% of the free thiamine and 20% of the bound thiamine are removed from the milk by the resin treatment. The copper content of the resin-treated milk was reduced approximately 23%.

The testing of nuclear weapons has contaminated the environment with radionuclides. Some of these radionuclides—strontium-89 (^{89}Sr), strontium-90 (^{90}Sr), iodine-131 (^{131}I), barium-140 (^{140}Ba), and cesium-137 (^{137}Cs)—enter the food chain of man and consequently present possible health hazards.

The potential health hazard of these radionuclides is determined primarily by the half life of the isotope and the degree to which the isotope is concentrated and retained in certain tissues. Consequently, ^{89}Sr, ^{90}Sr, and ^{131}I are the isotopes of primary concern in the contamination of the human food chain, because ^{90}Sr, with a half life of 27 years, and ^{89}Sr with a half life of 54 days, are very similar to calcium in metabolic patterns and concentrates in bones, while ^{131}I, with a half life of only 8 days, concentrates heavily in the thyroid gland. Because of the health hazards associated with the internal radiation from the disintegrating radiocontaminants, methods have been investigated for reducing the level of radioactivity in consumer milk.

Several laboratories have shown the effectiveness of cation exchange resins, in the sodium, potassium, or calcium form, in removing radiostrontium (*9–11*, *20*, *22*) and radiocesium (*20*) from milks labeled both in vitro and in vivo.

In most cases, the removal of up to 90% of strontium and cesium resulted in gross changes in the cationic composition of the milk (*10*, *11*, *22*, *25*) and, in some cases, changes in its physical and chemical properties, such as pH, titrable acidity, curd tension, and rennin coagulation time (*10*).

A recent report by Aarkrog and Rosenbaum (*1*) indicated that three successive resin treatments of strontium-85-labeled milk using batch techniques would remove 99.7% of the strontium but that preliminary data indicated a loss of certain vitamins. A later report from the same laboratory showed that thiamine, riboflavin, and vitamin B_6 were reduced by 60, 40, and 10 to 20%, respectively (*23*).

It was evident that such procedures, while capable of removing cation radionuclides from milk, would have practical limitations unless the physical appearance, organoleptic properties, sanitation, and nutritive qualities of consumer milk were preserved.

A method for removing cationic radionuclides from milk has since been developed with limited changes in cationic composition, physical appearance, and organoleptic properties of the milk (*12*, *21*). The ion exchange resin used in the development of this method was a sulfonated copolymer of styrene and divinylbenzene having a particle size range of 20 to 50 mesh, and a medium porosity. Several companies prepare such a resin for general-purpose commercial use. The resin-manufacturing techniques can be regulated to yield a desired degree of crosslinking (porosity) and a specified particle size range. Higher ion exchange capacities (about 5.0 meq. per gram of resin) can be provided in this type of copolymer than in the former phenolic condensation products (*19*). The resin is very durable in alkaline and acidic solutions and stable up to temperatures of 100° C. and higher. These properties recommend its use for treatment of food products. This type of resin is included in a list of ion exchange resins approved as safe for the treatment of foods when used under conditions prescribed by the Food and Drug Administration (*13*).

The assessment of the nutritive quality of milk from such a treatment (*12*) has been attempted with two approaches—chemical analyses, and feeding studies and

[1] Division of Radiological Health, U. S. Public Health Service, U. S. Department of Health, Education and Welfare, Rockville, Md.

[2] Dairy Products Laboratories, Eastern Utilization Research and Development, U. S. Department of Agriculture, Beltsville, Md.

[3] Present address, Veterans Administration Hospital, Coral Gables, Fla.

subsequent biological analyses. This communication reports the results of the chemical analyses conducted on milk processed for removal of cationic radionuclides.

Experimental

Fresh raw whole milk was divided into two lots. One lot served as the control and the other lot was used for the ion exchange treatment. The control milk for the study was prepared by standardizing the raw whole milk to 3.5% butterfat, pasteurizing at 161–5° F. for 15 seconds, and then homogenizing at 2000 p.s.i. The lot of raw whole milk designated for the ion exchange treatment was likewise standardized to 3.5% butterfat and then subjected to the procedure developed for removing cation radionuclides from milk by ion exchange resin techniques (*12*). The resin-treated milk (processed) was then pasteurized and homogenized in the same manner as the control milk.

Samples of the standardized control and processed fluid whole milk were analyzed for various nutritional components essential to experimental animals and man. The remainder of the milk of each treatment was concentrated to 30 to 40% total solids, cooled in an ice water tank to 50° F., and spray-dried with an incoming air temperature of 265° F. The resulting milk powder from each treatment was then air-packed in 25- to 50-pound quantities in polyethylene-lined drums, placed in cold storage at 4° C., and used for subsequent feeding studies, the results of which will be reported in another communication.

The effect of the resin-treatment process on the chemical composition of milk was investigated by considering first some of the gross chemical analyses routinely used as indicators of the composition of milk, such as total solids, butterfat, total protein, and ash, with a physical examination of the flavor quality (Table I), followed by detailed chemical determination of specific vitamins, minerals, and fatty acids. All determinations were made on standardized fluid whole milk, and all the data from the various determinations on the processed milk have been corrected for a dilution of approximately 5% owing to the resin treatment process, primarily from acidification with citric acid and later neutralization with potassium hydroxide.

Total solids, per cent butterfat, and per cent ash were determined according to the methods reported by Jacobs (*17*); the Majonnier apparatus was used in the total solids and butterfat assay. The per cent total protein was determined by the micro-Kjeldahl method according to AOAC procedures (*2*). The organoleptic quality of milk was determined by a 10-judge panel using a modified flavor scoring guide approved by the American Dairy Science Association. Carotene and vitamin A were determined on a lipid extract of the control and processed milk using the Carr-Price reaction (*8*). Thiamine was determined by the thiochrome method of Hennessy (*16*) with a slight modification in the enzymatic hydrolysis as described by Simpson and Chow (*24*). Five milliliters of a solution containing 5 grams of takadiastase and 5 grams of pepsin in 100 ml. of 2.5*M* sodium acetate (pH 4.0 to 4.5) were added to each reaction flask and incubated at 50° C. for 3 hours instead of using takadiastase alone and incubating for only 2 hours. The enzymatic hydrolysis was omitted for the determination of free thiamine. Bound thiamine was calculated by subtracting free thiamine from total thiamine.

Table I. Gross Chemical Analyses

Determination, %	Milk	
	Control	Processed
Total solids	12.79	12.41
Butterfat	3.63	3.50
Total protein	3.13	3.15
Ash	0.70	0.80
Flavor quality determined by physical examination	36.6	35.8

Riboflavin was determined both fluorometrically (*18, 27*) and microbiologically, using *Lactobacillus casei* (ATCC 7469) as the test organism, according to the procedure of Snell (*26*). The microbiological procedure for the determination of niacin, pantothenic acid, and folic acid was essentially as described by Bakerman *et al.* (*5*), except that the test organism for folic acid was *Lactobacillus casei* (ATCC 7469) instead of *Streptococcus faecalis* (R-ATCC 8043). The test organism for niacin and pantothenic acid was *Lactobacillus arabinosis* (17-5 ATCC 8014) as previously reported (*5*). The microbiological determination of vitamin B_6 (pyridoxine, pyridoxal, and pyridoxamine) was made according to the method of Toepfer and Lehmann (*28*) using *Saccharomyces carlsbergensis* (ATCC 9080) as the test organism. Vitamin B_{12} was extracted by the method of Gregory (*15*), except that the samples were steamed for 40 minutes instead of autoclaved for 10 minutes. The vitamin B_{12} concentration of the extracts was then determined microbiologically using *Escherichia coli* as the test organism according to the method of Burholder (*7*).

The fatty acid composition was determined on a lipid extract of the milks. The lipid was saponified and the fatty acids were methylated in methanol–benzene in the presence of boron trifluoride (*6*). The methyl esters were then determined by gas-liquid partition chromatography. The chromatography column and conditions were described by Bieri (*6*).

Sodium, potassium, magnesium, and calcium were determined by flame spectrophotometry as described by Wenner (*30*). The colorimetric method of Fiske and Subbarow (*14*) was used for phosphorus determination except that the sample was prepared according to the microchemical AOAC method (*3*). Phosphorus was also determined by the spectrographic AOAC method (*4*), using a Bausch & Lomb medium quartz spectrograph. Iron and copper were determined by the AOAC method (*4*), and read on a medium quartz Bausch & Lomb spectrograph.

Results and Discussion

The entire process of removing radiocontaminants from milk must also preserve the physical appearance, organoleptic properties, sanitation, and nutritive qualities of milk. Milk has been subjected to similar treatments with ion exchange resins, and in some cases, the resins that were used to alter the electrolyte balance of bovine milk were identical to the cation resins used in the procedure described for removing radionuclides (*12*). The previous reports (*29*, *31*) on alterations in electrolyte balance of milk have been primarily for producing "low-sodium" milk and human infant formulas. The chemical composition of "low-sodium" milk produced by ion exchange techniques compared favorably with whole milk in many nutrients, but thiamine, niacin, and vitamin B_{12} were reduced by 50% while calcium and vitamin B_6 were reduced by 75% (*31*). The potassium content was increased to almost twice that of whole milk and the ash content increased approximately 12% (*31*), probably because the sodium of milk exchanges for potassium on the resin.

One of the most important contributions in the method of Murthy *et al.* (*21*) for removing radionuclides from milk was maintaining the normal major cation composition (calcium, magnesium, sodium, and potassium) of milk by previously charging the resin with a chloride mixture of these cations. Although the potassium content of milk is essentially unaltered in the resin treatment, the subsequent use of 0.75*M* potassium hydroxide (40 ml. per liter of milk) to adjust the pH to 6.6 increases the potassium content approximately 80%. No adverse effects from the increased potassium content would be expected, since it is within the range (3 to 5 grams per day) of normal physiological intake for adults (*31*). Acidification of the milk with 16 to 18 ml. of 0.75*N* citric acid per liter of milk increased citric acid content to almost twice that of whole milk (calculated value).

The results of this study indicate no statistically significant change in either the flavor score or the concentrations of total solids, butterfat, or total protein when processed milk was compared with control milk (Table I). However, the per cent ash of the processed milk was increased 14% (Table I), a significant difference ($P < 0.05$; P means probability). The analysis of the long-chain fatty acid components (Table II) of the milks showed no significant alterations in fatty acid distribution as a result of the cation resin treatment.

In retrospect, determination of the short-chain fatty acids might have been a better indicator of any changes in butterfat composition, but it seems unlikely that such a resin treatment of milk would result in major changes in fatty acid composition, particularly since very little free fatty acid is found in milk, while most fatty acids exist in the form of triglycerides or neutral lipids. Consequently, the fat globules of the butterfat would be expected neither to absorb on the cation columns nor to hydrolyze to the free fatty acids under these conditions.

Mineral concentrations (Table III) were generally in accord with those of Murthy *et al.* (*21*), except for the significant increase in potassium concentation ($P < 0.01$) due to neutralization of acidity with potassium hydroxide. The concentrations of calcium, magnesium, sodium, and phosphorus showed some variation between the control and processed milks, but no differences were statistically significant ($P > 0.05$). Of the trace minerals determined, the concentration of iron in the two milks was unaltered while the concentration of copper in the processed milk was decreased slightly but not significantly compared with the control milk (Table III).

The carotene, vitamin A, riboflavin, pantothenic acid, folic acid, and vitamin B_{12} contents of the processed milk were not significantly changed, compared with control milk (Table IV). The thiamine, niacin, and vitamin B_6 contents of the processed milk were reduced by 50, 27, and 15%, respectively, compared with the control milk; the thiamine and niacin contents were significantly decreased ($P < 0.01$). The data presented here on thiamine and vitamin B_6 reduction agree very well with those of Aarkrog and Rosenbaum (*1*). However, this study indicates a 27% reduction in niacin content of processed milk, while White (*31*) reported a 50% reduction.

The vitamin B_6 content of the processed milk is reduced by only 15%, even though the ion exchange resin used for removing cation radionuclides is identical to the resin used by Toepfer and Lehmann (*28*) for chromatographic separation of the three forms of vitamin B_6 (Table IV). As a possible explanation, the chromatographic procedure calls for autoclaving 5 hours at

Table II. Fatty Acid Composition

Fatty Acid[a]	Milk, % Control	Milk, % Processed
10:0	Trace	Trace
12:0	3.2	1.9
14:0	15.7	14.3
15:0	1.6	1.5
16:0	42.1	44.0
16:1	1.1	1.1
18:0	9.5	10.2
18:1	24.8	25.0
18:2	1.4	1.4
18:3	1.0	1.0

[a] Number preceding colon is carbon chain length, number following colon is double bonds.

Table III. Mineral Analyses

Element	Milk, Mg. per Liter Control	Milk, Mg. per Liter Processed
Ca	1242.00	1187.00
Mg	107.00	110.00
Na	479.00	491.00
K	1391.00	2503.00
P	855.00	874.00
Fe	0.75	0.74
Cu	0.23	0.16

Table IV. Vitamin Analyses

Compound	Milk, μg. per Liter			
	Control		Processed	
Carotene	164.5		157.5	
Vitamin A	47.3		45.5	
Thiamine (total)	275.0		140.4	
Free thiamine		137.5		19.3
Bound thiamine		137.5		121.1
Riboflavin	1250.0		1340.0	
Pantothenic acid	2580		2670.0	
Niacin	840.0		610.0	
Folic acid	9.4		8.6	
Vitamin B_{12}	4.6		4.4	
Vitamin B_6 (total)	390.0		330.0	
Pyridoxine		20.0		10.0
Pyridoxal		290.0		250.0
Pyridoxamine		80.0		70.0

15-pound steam pressure; this liberates the organically bound forms of vitamin B_6, which is then adsorbed onto the ion exchange resin at pH 4.5. The vitamin B_6 in the processed milk is organically bound in a naturally highly buffered solution and passed over the resin at pH 5.4.

The data on the removal of free and bound thiamine indicate that organically bound forms are unavailable for adsorption (Figure 1). Since the findings of this and other laboratories (*1*, *31*) have demonstrated that the thiamine content of milk exposed to cation exchange resin is reduced 50%, the authors decided to investigate more closely the two forms of thiamine. As can be seen in Figure 1, the quantities of free and bound thiamine are about equally divided in the control milk. After 2 to 6 bed volumes (BV) of milk had passed through the column, 61% of the total thiamine (93% of the free; 36.5% of the bound) was removed; after 10 to 14 bed volumes 47% of the total thiamine (85% of the free; 18.5% of the bound) was removed; after 24 to 28 bed volumes, 41% of the total thiamine (83% of the free; 8% of the bound) was removed. According to Murthy *et al.* (*21*), a constant removal of 94 ± 2% of ^{85}Sr and 92 ± 2% of ^{140}Ba occurred up to 30 resin bed volumes of milk, beyond which a gradual decrease was observed. At this point, the resin columns are regenerated for continued high percentage removal of cation radionuclides. The data (Figure 1) indicate a similar point of resin exhaustion for thiamine removal. Approximately 68.5% of the thiamine removal was recovered by eluting the resin column with 1 liter of 25% KCl in 0.1*N* HCl, the solution used to elute adsorbed thiamine from Decalso columns in thiamine determinations (*16*). Perhaps a greater percentage of thiamine could be recovered with greater elution volumes or different solvents, but this indicates that a considerable amount of thiamine is adsorbed rather than destroyed during the process.

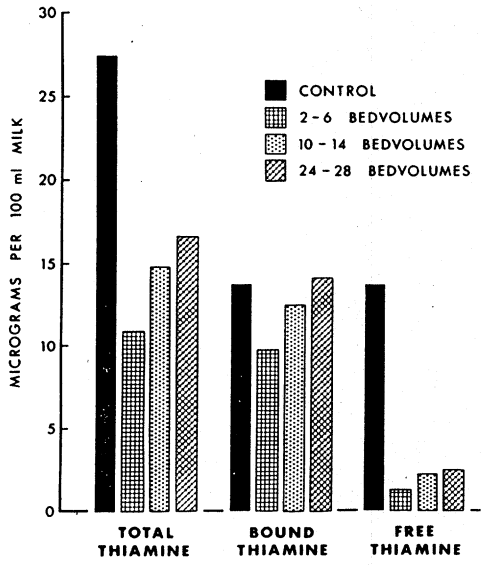

Figure 1. Effect of strontium-removal process on thiamine content of milk

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